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Characterization of typical aquatic humic substances in areas of sugarcane cultivation in Brazil using tetramethylammonium hydroxide thermochemolysis

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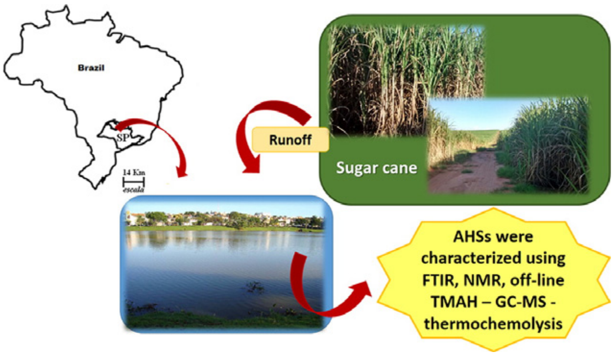
HIGHLIGHTS

- AHSs differ from one environment to another depending on land use and occupation.
- AHSs extracted from a river in a typical region of sugarcane cultivation.
- AHSs from the sugarcane area are influenced by the soil use and occupation.
- AHSs contain lignin derivatives, fatty acid methyl esters and others.
- Lignin was observed with greater intensity at the period of sugarcane harvest.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic humic substances (AHSs) differ from one environment to another depending on land use and occupation. In addition, the effects of planting sugarcane on AHSs are not well known. Thus, the aim of this study was to characterize AHSs extracted from a river in a typical region of sugarcane cultivation during dry and rainy seasons. The main characteristics of the AHSs were obtained using Fourier transformation infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and off-line pyrolysis coupled with gas chromatography and mass spectrometry (off-line tetramethylammonium hydroxide (TMAH)-GC–MS-thermochemolysis). The FTIR and NMR results were used to infer that no distinctions occurred between the sampling periods. The samples were composed of aromatic groups that were potentially associated with the presence of residual vegetable materials (lignin). The results of the off-line TMAH-GC–MS-thermochemolysis indicated that the structures of the AHSs had uniform compositions that were rich in fatty acid methyl esters (FAMEs), polysaccharide derivatives, aliphatic biopolymers derived from plants, long hydrocarbon chains, branched alkyl groups and methylene carbons. Thus, the results showed that the AHSs obtained from the sugarcane cultivation area during the crop period mainly consisted of resistant aliphatic hydrocarbons, which are derivatives of lignin and FAMEs in compounds rich in humic acid. Therefore, we concluded that sugarcane cultivation produces changes in AHSs because greater amounts of lignin derivatives were observed during the dry season, corresponding to sugarcane cultivation.

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1. Introduction

Aquatic humic substances (AHSs) are important in the environment because they contain complex aromatic rings and reactive functional groups that allow for the adsorption of pollutants, especially metals. Humic substances (HSs) mainly form in the environment from the decomposition and transformation of allochthonous and autochthonous biomolecules from plant and animal residues (Ryan, 2000; Rocha et al., 2004; Filella et al., 2005; Rodríguez and Núñez, 2011). Land use, occupation of the soil, seasonality, climate, and other factors influence the formation of HSs. In addition, these factors not only influence the composition of the HSs but also the types of processes that occur during their humification, which result in different structures (Cheng, 1977; McKnight et al., 1985; Sargentini et al., 2001; Alberts and Takács, 2004; Batley et al., 2004; Filella et al., 2005; Azevedo and Nozaki, 2008; Esteves et al., 2009). The bioavailability of metals and organic pollutants is affected by dissolved organic matter because the bond between the contaminant and organic matter, especially redox processes involving quinone radicals present in the structure, will result in the accumulation of organic macromolecule contaminants and toxic organics in aquatic environments (Kukkonen et al., 1990; Akkanen et al., 2012).

Characterization of AHSs can be used to determine which functional groups are responsible for their complexation with metals and other pollutants. For this analysis, various researchers have employed different analytical techniques, including NMR, FTIR spectroscopy, Fluorescence spectroscopy and off-line TMAH-GC–MS-thermochemolysis (Martin-Neto et al., 1996; Peuravuori et al., 2002; Sanches et al., 2007; Vieyra et al., 2009; Rodríguez and Núñez, 2011; Ziegelgruber et al., 2013). Although FTIR is a qualitative technique, it provides considerable information regarding the main functional groups that are present in the structure of HSs. This information can be used to infer the possible macromolecular structure of HSs (Spaccini and Piccolo, 2009; Rodríguez and Núñez, 2011). The FTIR spectra from HSs show bands that represent the stretching vibrations of different functional groups that are present in HSs (Filella et al., 2005; Spaccini and Piccolo, 2009; Giovanela et al., 2010; Rodríguez and Núñez, 2011).

Furthermore, ^{13}C NMR has been used to provide structural information regarding the conformational characteristics of the carbon nuclei in the sample. In addition, NMR can be used to estimate the relative amounts and types of aliphatic and aromatic carbon and the presence of phenolic and carboxylic groups in the structure of HSs. This information is obtained by integrating the peaks in specific regions of the spectrum to obtain information regarding the molecular structure and its origin (Martin-Neto et al., 1996; Esteves et al., 2009). Off-line TMAH-GC–MS-thermochemolysis has been established as a sample pre-treatment method that is used before characterizing the chemical structures of the complex and intractable materials. Each of these techniques has been used to characterize the molecular compositions of HSs (Challinor, 2001; Jarde et al., 2003; Durand et al., 2005; Spaccini and Piccolo, 2007, 2009; Shadkami and Helleur, 2010; Ohra-Aho et al., 2012). In the TMAH-GC–MS-thermochemolysis technique, the methylation of the esters and ethers present in the sample increase the thermodynamic stability and chromatographic detection of the method when a break occurs, providing essential information, such as the number of key methoxide groups in the structure with ester bonds (C–O) and amine bonds, among others, that are linked to β O-4 lignin, methylated acids, alcohols, and other phenolic monomers (Spaccini and Piccolo, 2007, 2009). Thus, this technique is capable of verifying the molecular structure of HSs (Pulchan et al., 2003; Nebbioso and Piccolo, 2011).

Rivers within the Turvo Grande watershed are of great interest for aquatic humic studies because the soil and land use has changed in recent decades. Pastures have been replaced with sugarcane plantations which are associated with a lack of riparian vegetation in aquatic bodies. It is important to note that this area includes the largest sugarcane cultivation area in the state of São Paulo, accounting for 52% of sugarcane production in Brazil (CONAB, 2012). The Preto River is one of the most important tributaries in the Turvo/Grande watershed, having a high water availability, and flows into the Rio Grande to form the Paraná River, which is the second largest South American river (CBHTG, 2014). Several studies have been conducted in this aquatic body. AHSs from Preto River display characteristics of recently degraded organic matter. This finding was confirmed by the presence of predominantly aliphatic structures with a low degree of humification (Melo et al., 2012). A characterization of HSs from sediments of the Preto River using off-line TMAH-thermochemolysis-GC–MS showed contributions from compounds rich in lipids and fatty acid methyl esters (FAMEs), plant waxes, plant biopolyesters and a large amount of FAMEs that are related to the large amount of HA in the structure. The analyses also revealed a higher contribution of lignin derivatives in the structure, which can be attributed to greater intensity of sugarcane cultivation in this region and the lack of riparian vegetation in this location (Tadini et al., 2015). Considering the importance of improving our understanding of the effects of sugarcane and seasonality on the characteristics of AHSs, the main objective of this study was to characterize AHSs using FTIR, NMR, and off-line TMAH-GC–MS-thermochemolysis.

2. Materials and methods

2.1. Collection and extraction of AHSs

This study was conducted in the Preto River area, a typical area in the northwest region of São Paulo that is known for its sugarcane production. The study area is characterized by a tropical climate with an average temperature of 25 °C and well-defined seasons. Surface water samples were collected in a location representative of the sugarcane cultivation region (S20°48′40.94″ W49°21′13.62″) during the dry season (April to September 2011) and rainy season (October 2011 to March 2012) and were shipped to the laboratory for analysis. The temperature, pH, conductivity, turbidity and dissolved oxygen parameters were measured in the field using portable meters (Hanna HI991300 and Hanna HI9146-04) that were calibrated in the laboratory. The dissolved organic carbon (DOC) content was determined using a total organic carbon analyzer (Shimadzu TOC-VCSN). Additional details regarding the sample analyses can be found in the study conducted by Melo et al. (2012). The collection of surface water samples for SHA extraction was performed according to the recommendations of USEPA (2000, 2007) and method 1669 (USEPA, 1996). The extraction of AHSs was performed according to the recommendation of the International Humic Substances Society (IHSS) employing a polymer resin for the retention of humic and fulvic acids (Thurman and Malcolm, 1981). The humic and fulvic acid contents were quantified after the solubilization of a known amount of AHSs from the dry and rainy periods using chloridic acid until the pH was less than 2.0. After the precipitation step, each sample was centrifuged at 4000 rpm for 30 min.

2.2. Characterization of the AHSs

2.2.1. FTIR

To characterize the AHSs using FTIR, 1.0 mg of the AHS samples from the from dry periods was mixed with 3.0 mg KBr (a 1:3 ratio of sample: KBr). Spectra were obtained from 4000–450 cm^{-1} with a resolution of 4 cm^{-1}, and 8 scans were collected using a PE Spectrum-One device equipped with a diffuse reflectance accessory (PerkinElmer, adapted from Spaccini and Piccolo, 2009).

2.2.2. ^{13}C-CPMAS-NMR

Fine-powdered composite samples were analyzed by solid-state NMR spectroscopy (13C CPMAS NMR) on a Bruker AV300 Spectrometer equipped with a 4 mm wide-bore MAS probe. The NMR spectra were